A PRELIMINARY ACCOUNT OF THE CHROMOSOMES IN THE EMBRYOS OF ANASA TRISTIS AND DIABROTICA VITTATA.

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It was suggested to the writer, in 1912, by Prof. E. G. Conklin that a comparative study be made of the chromosomes in the somatic cells of certain insects, as contrasted with the sex cells. The first material was collected during the following summer, and included embryos of *Anasa tristis* and *Diabrotica vittata*. Material of a number of other forms, not restricted to the Insecta has since been collected and examined to some extent. The results obtained from the latter, together with a more extended account of the forms briefly described here, will be discussed at greater length in another paper.

Embryos of *Anasa tristis* were obtained covering a period of development from the incomplete blastoderm stage up to about the time of hatching. Some difficulty was experienced in obtaining the eggs of *Diabrotica vittata*, but fortunately the stages obtained extend from the second maturation division up to the segmentation of the embryo.

Anasa tristis.—In 1906, Wilson in his "Studies on Chromosomes," Part III., gave the results of his work on the spermatogenesis of *Anasa tristis*. There are twenty-one and twenty-two chromosomes in the spermatogonia and oögonia, respectively. Of the twenty-one chromosomes in the spermatogonia, twenty can be paired. There is, here, a marked differentiation in the size of the chromosomes. In each plate there are three large or macrochromosomes, and two small or microchromosomes. The unpaired or x-chromosome (idiochromosome; accessory chromosome) is one of these three largest chromosomes, although it cannot be exactly determined which one it is. The two microchromosomes have been termed the m-chromosomes. In the second maturation division the x-chromosome moves un-

divided to one pole so that one half the number of the spermatids receive eleven, the other half, ten chromosomes. Each spermatid also receives one *m*-chromosome.

In the oögonial group there are twenty-two chromosomes, which can be arranged in eleven pairs. Of these, four are macro- and two are microchromosomes. Wilson was able to count the chromosomes in the ovarian follicle-cells. These were found to be identical in number with the chromosomes in the oögonia. He says, however, that "not infrequently the number of chromosomes is much greater, and the same is true of the nuclei of the investing cells of the ovary, of the oviduct and the fat-body. In the male similar multiple groups are not uncommon in the interstitial and investing cells of the testis. Only in a single case have I succeeded in gaining a clear and complete view of such a group; but this one case suffices to give, with a great degree of probability, the explanation of the increased number of chromosomes. In this case every chromosome is exactly twice the oögonial number, namely 44." This figure, which he gives, is from a cell toward the periphery of a larval ovary, and shows eight macro- and four microchromosomes, twice the number of these particular chromosomes found in the oögonia. He suggests that the chromosomes have divided once without a corresponding division of the cell-body, and he thinks it probable that an increase in number of chromosomes in these particular cells is always due to a process of this kind.

Wilson also states that although he was unable to obtain perfect preparations of mitoses in other tissues, he is able to assert that in the ectodermal cells of the larva the number of chromosomes is "approximately the same as in the oögonia," and that nowhere else than in the described cases did he obtain a doubling of the number. He concludes that this multiplicity is due, perhaps, either to the fact that the cells in question are degenerating, or that they are highly specialized.

In 1910, Morrill published his observations on the chromosomes in the oögenesis, fertilization, and cleavage of certain Coreid Hemiptera, among them *Anasa*. He found that the oögonial groups in *Anasa* contain twenty-two chromosomes, including four macro- and two microchromosomes. The reduced number in

the matured egg he found to be eleven. The chromosomes in the reduced groups, either in the polar body or the egg, showed the same relative size differences as the corresponding pairs in the oögonia, and the chromosomes in the matured ova correspond, in general, to those of a sperm bearing the *x*-chromosome. The chromosome groups, which were counted in the embryonic mitoses of *Anasa* were from incomplete blastoderm stages. The embryos were found to be of two kinds: one containing twenty-one chromosomes, the other twenty-two. One case was reported of twenty-three chromosomes, but this Morrill suggests may be due to an accident in technique. The two different chromosome groups, those having twenty-one and twenty-two, correspond, respectively, to the groups found in the spermatogonia and the oögonia. There are the same size differences, and the same number of macro- and microchromosomes. He states that the chromosomes in general are more elongated in the embryonic mitoses than in those of the germ cells, which makes the task of pairing them extremely difficult, if it is at all possible, except in the case of the macrochromosomes and the two *m*-chromosomes.

The results, which the present report embodies, were obtained, in the case of *Anasa*, from a study of the somatic mitoses in several stages of development. Although mitoses are abundant in all the tissues of the embryos, few are sufficiently favorable for even tentative counts, still fewer for actual counts. In no case has it been possible to obtain more than seven counts from one individual, while three counts are more nearly the average. The chromosomes, especially in the metaphase groups, are crowded together, and the possibility of counting them at all depends chiefly on the extent of destaining.

At first some rather startling results were obtained. Some counts revealed a number as low as fourteen chromosomes in one plate, while others ran up to twenty-four in number, and there seemed to be no number characteristic of any one tissue, though the average varied around eighteen and twenty. When, however a large number of series was examined, and the exact point of optimum destaining was acquired, the results tabulated showed that the seemingly aberrant counts were wrong, and that they might be due to any of the following reasons; (1) counting early

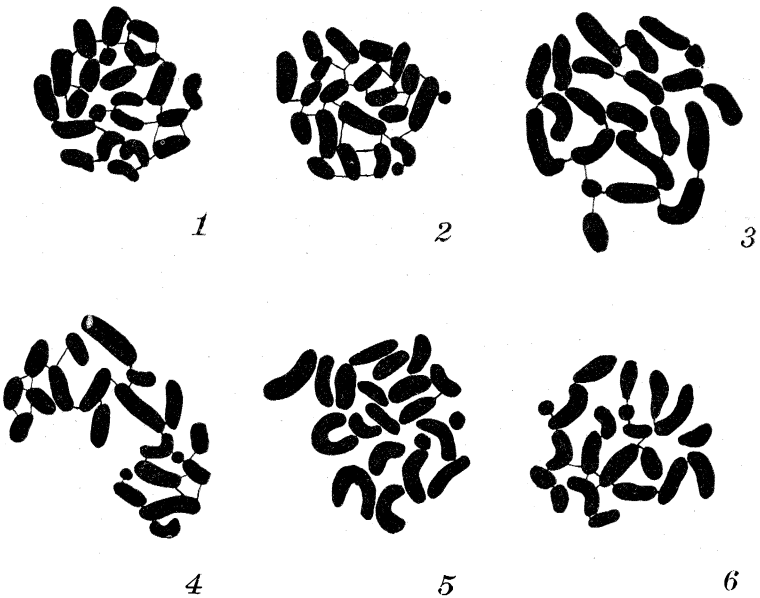
prophases, where the chromosomes lie at different levels, and are crowded and overlapped; (2) counting plates, one or several chromosomes of which lay in an adjoining section (though this does not mean that such figures are necessarily valueless); (3) some chromosomes were torn out of the section; (4) one or more chromosomes had been cut by the microtome knife, and the resulting ends or segments appeared in the next section; (5) the destaining of the slide had not progressed far enough to differentiate, one from another, some of the chromosomes lying quite close together.

Approximately one hundred groups of chromosomes from different tissues, and from a large number of embryos, have been drawn and counted. The stages, extending from the incomplete blastoderm stage up to about the time of hatching, which have been examined, are at a period of more or less high differentiation, when any chromosomal differentiation would most likely be apparent, if this is a regular concomitant of cell differentiation. The results so far show most conclusively that there is no such differentiation in the number of the chromosomes in *Anasa*, and that the embryos are of two classes as to their chromosome number, one having twenty-one, and the other twenty-two chromosomes in all the somatic cells. Moreover, the same general size differences in the chromosomes may be observed in these comparatively late stages of development as are apparent in the spermatogonia and the oögonia.

The accompanying figures serve to illustrate these points. The three upper figures (Figs. 1, 2 and 3) are from embryos having twenty-two chromosomes, and are at a stage of development where the limbs are quite elongated and the embryo as a whole has not shortened up. Figs. 1 and 2 are from the hypodermal layer of the antenna, and are from the same embryo. In both figures it will be noted that there are four chromosomes larger than the others, though this difference in size is perhaps clearer in Fig. 2. In each of the figures, again, the two *m*-chromosomes appear. Fig. 3 is from a cell in the cerebral ganglion of another embryo. These cells are much larger than any others in the embryo, consequently the chromosomes are much larger. The *m*-chromosomes are easily seen, and one pair of chromosomes is

distinctly larger than the others. The second pair of macrochromosomes is somewhat more difficult to make out.

The three lower figures (Figs. 4, 5 and 6) are from embryos with twenty-one chromosomes. Figs. 4 and 6 are from mesoderm cells of two different embryos, at a stage when the limb buds are first appearing. Fig. 5 is from a cell in the cerebral ganglion



Anasa tristis. Initial magnification of 4,800 diameters, reduced one third. Figs. 1 and 2, hypodermal layer of the antenna, 22 chromosomes. Fig. 3, cerebral ganglion cell, 22 chromosomes. Fig. 4, mesoderm cell, 21 chromosomes. Fig. 5, cerebral ganglion cell, 21 chromosomes. Fig. 6, mesoderm cell, 21 chromosomes.

of another individual, at a much later stage of development. In each of these figures the *m*-chromosomes are easily distinguishable, and it can be seen that there are two paired and one unpaired macrochromosome, particularly well shown in Fig. 4. Although no attempt has been made to pair the chromosomes, in several of the figures shown it would be quite possible to do so.

In all the cases studied the twenty-one chromosome groups generally show quite clearly three chromosomes larger than the rest, while the twenty-two chromosome groups show four such chromosomes. In most of the figures the *m*-chromosomes stand

out clearly. In a few cases it is difficult to make them out, owing to the fact that they lie very close to a larger chromosome.

Of course a difference in the general size of the chromosomes as a whole is to be found, and it is probable that no two groups would appear exactly alike. The size of the chromosomes in the metaphase groups is directly proportional to the size of the cytoplasmic body. In the metaphase groups there is a longitudinal splitting of each chromosome. In no case has a transverse division been observed here, nor has there been observed a doubling in the number of chromosomes in this phase, such as Wilson describes in the follicle cells.

Diabrotica vittata.—The spermatogenesis of *Diabrotica vittata* was worked out and published in 1907 by Miss Stevens. In the spermatogonial divisions she found twenty-one chromosomes of various sizes and shapes. The *x*-chromosome which she figures is one of the medium-sized chromosomes. The other twenty she was able to pair. In the second spermatocytic division the *x*-chromosome appears as a more or less rounded body, which passes undivided to one of the poles of the spindle. There are no such clear cut size differences as are found in *Anasa*.

The results which I have obtained from a study of the eggs and early embryos of *Diabrotica*, though somewhat meager it is true, clearly support the same conclusion as was reached in the case of *Anasa*, namely that the embryos are of two classes, one having twenty-one, and the other twenty-two chromosomes in all the mitoses.

Some clear cut figures were obtained in the maturation of the egg. The anaphase of the first maturation division revealed eleven dyads. These in turn divided, leaving eleven monads in the fully matured egg. This brings the maturation of the egg of *Diabrotica* into line with that described for other insects.

In conclusion it may be said that in neither *Anasa* nor *Diabrotica* were there any numerical differences between the gonial and the somatic chromosomes, such as have been described in the rabbit by Winiwarther, and more recently, in man by Wieman, in *Osmia cornuta* by Armbruster, and in *Diaptomus* by Krimmel.

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